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FORCED DEGRADATION STUDIES AND ASSESSMENT OF DEGRADATION PRODUCTS OF IMEGLIMIN HYDROCHLORIDE USING LC-ESI/APCI-MS

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ABSTRACT

Forced degradation subjects drug substances to stress conditions, producing degradation products that are crucial for evaluating stability under accelerated conditions. Imeglimin hydrochloride is a commonly prescribed medicine for managing type 2 diabetes mellitus. This study details the forced degradation experiments and LC-MS analysis of degraded products of Imeglimin Hydrochloride under various stress conditions, offering insights into its stability and providing information on fragmentation pathways and major degradation products. The optimized mobile phase for the LC-MS method was composed of 10 mM ammonium format buffer (pH 3): methanol with a ratio of 75:25 v/v, obtained on an Xtimate C-18 column (flow rate of 0.8 ml/min), with 234 nm as the specific detection wavelength for Imeglimin Hydrochloride. The drug was subjected to stress conditions including photolysis, hydrolysis, thermal, and oxidative stress conditions. After various stress conditions were applied, the complete stability pathways and effects of these stress conditions were examined. Imeglimin hydrochloride underwent degradation when exposed to oxidative conditions at room temperature and basic conditions at elevated temperatures. However, specific degradation products were not found under acidic, thermal, and photolytic stress conditions. By comparing predicted and observed fragmentation patterns and identifying base peaks, LC-MS analysis of Imeglimin Hydrochloride revealed two new degradation products, DP1 (m/z 160.2) and DP2 (m/z 118.0) under oxidative and basic stress conditions, respectively. The developed method was validated as per the guidelines of analytical method validation.

Keywords: Forced Degradation, Imeglimin Hydrochloride, Liquid Chromatography-Mass Spectrometry, Stability Studies, Stress Testing.

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INTRODUCTION

Diabetes mellitus is a major chronic metabolic disorder observed by consistently increased blood sugar levels because of improper insulin action and inadequate insulin production. This condition not only has significant health impacts but also raises various health issues like elevated blood pressure, altered cholesterol levels, atherosclerosis, and increased platelet aggregation.[1,2] Numerous treatments are available for Type 2 Diabetes Mellitus (DM), including combination therapy, bariatric surgery, lifestyle modifications, and insulin therapy. Imeglimin hydrochloride is the first oral tetrahydro-triazine compound of the "glimin" family developed for treating type 2 DM. It stands out due to its dual mode of action, which sets it apart from other hypoglycemic medications. IMEG works by enhancing insulin sensitivity and restoring proper function to pancreatic β-cells, offering a unique approach to managing type 2 DM. [3,4,5] The structural formula of Imeglimin hydrochloride is depicted in Fig.-1 below:

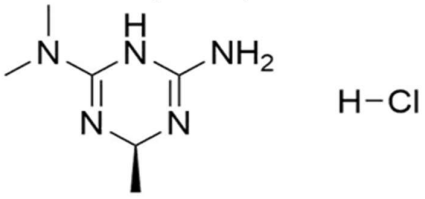

Fig.-1: Structure of Imeglimin Hydrochloride

IMEG primarily acts on the mitochondria of aerobic cells, enhancing their bioenergetics. It also helps to prevent apoptosis of pancreatic β-cells and preserves their function.[6,7] Forced degradation studies are essential for understanding a drug's intrinsic stability and potential degradant formation, which is crucial for developing a stable formulation.[8] Utilizing LC-MS with various resolutions and ionization techniques enables the evaluation of proposed structures of unknown impurities and helps determine

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the fragmentation pathways of potential degradants.[9,10] The literature survey has documented several methods for determining IMEG, including UV spectroscopy and RP-HPLC.[11,12] However, there is a notable research gap in forced degradation studies and the evaluation of degraded products for IMEG. Therefore, the current research aims to comprehensively elucidate the degradation pathway of IMEG after applying various stress conditions and concluding the possible structure of major degradation products of IMEG.

EXPERIMENTAL

Material and Methods

Pure IMEG was generously provided as a gift sample by Torrent Pharmaceuticals Limited (Ahmedabad, India). All the necessary reagents for the research were sourced from registered suppliers, ensuring they were of HPLC grade or analytical grade quality. A Shimadzu HPLC system (LC-2050C 3D, Japan) equipped with an autosampler, quaternary gradient pump, and inline degasser was used for the overall research work. Separation of closely eluted products due to degradation was achieved using an Xtimate C-18 column, with peak purity confirmed by the PDA detector. Identification and characterization of DPs were performed using an LC-MS single mass spectrometer (Agilent 1260 Infinity-II) coupled with an MM+ES+APCI for high sensitivity, advanced analysis, and accurate results.

General Procedure

The HPLC system, consisting of a single pump, PDA detector, autosampler, degasser, and system controller (Agilent, Santa Clara, California, USA), was used. All compounds were separated by a reverse phase Xtimate C18 column (150mm x 4.6mm x 5µm) using 10 mM ammonium format buffer (pH 3): methanol with a ratio of 75:25 v/v as an optimized mobile phase at 234nm with the flow rate of 0.8ml/min for 15minutes as a runtime. The injection volume was 10 µL. The data were acquired and processed using open lab chem station software. High-resolution mass spectrometry data were obtained mass spectrometer equipped with a multimode APCI+ESI as an ionization source. The working standard solution of IMEG was prepared using a 75:25 (v/v) mixture of water and acetonitrile to achieve a stock solution concentration of 1000 µg/ml. Further dilutions were prepared using the same stock solution. A blank solution (diluents) was prepared by following the same procedure but omitting the drug substance.

Forced Degradation Studies of IMEG

IMEG was subjected to stability testing under various stress conditions to evaluate its chemical stability. The drug underwent controlled exposure to acidic, oxidative, basic, thermal, and photolytic conditions across various temperature settings. Changes in purity were then compared to a control sample. The specific optimized conditions for the forced degradation of IMEG are detailed in Table-1 below.

Table-1: Optimised Stress Conditions for Force Degradation Studies of IMEG

Stress conditions	Respective reagent/ Equipment	Exposure	Duration
Acid	1N HCl	80ºC	24 Hours
Base	0.1N NaOH	80ºC	24 Hours
Oxidation	1% H_2O_2	Room Temperature	Immediate injection of freshly prepared sample
Photolysis	1mm Petri-dish	Ultra-violet light	24 Hours
Thermal	Oven - Dry heat	75ºC	24 Hours

Analytical Method Validation

The developed chromatographic method was subjected to validation for various parameters of analytical method validation, including linearity, specificity, accuracy, precision, LOD, and LOQ, following the standard procedure outlined in ICH Q2 (R2). Since the primary focus of the research article was to determine the forced degradation and stability pathway of IMEG, these parameters were specifically evaluated to ensure the reliability of the analytical method.

Mass Spectrometric Conditions

The mass spectra were obtained in atmospheric pressure chemical ionization and electron spray ionization in positive mode detection. The ion source conditions were set with the dry gas temperature at 300°C. The pressure of the nebulizer gas was set to 1811, with the capillary voltage at 2000 V and

the corona current at 5 nA. The vaporizer temperature was set at 200°C. Pure nitrogen served as the shielding and collision gas, with zero air employed as the nebulizer.

RESULTS AND DISCUSSION

A variety of mobile phases were tested to obtain an MS-compatible mobile phase for LC-MS method development. Figure-2 displays the chromatogram of the standard IMEG under optimized chromatographic conditions which shows the retention time of IMEG as 3.776 minutes.

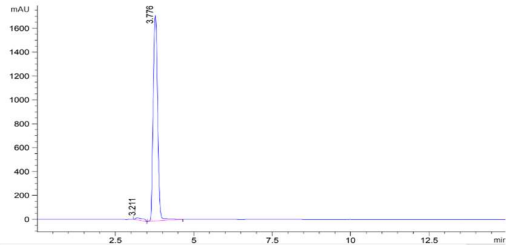

Fig.-2: Chromatogram of standard Imeglimin hydrochloride

Forced Degradation Studies of IMEG

As mentioned in the methodology section, stability studies of IMEG were done under various stress conditions like hydrolytic, oxidative, photolytic, and thermal. Significant degradation was noted under basic and oxidative stress conditions in which the additional degradants peak was clearly observed in both the chromatograms of basic and oxidative stress conditions respectively. However slight decrease in peak intensities was observed with photolytic, thermal, and acidic stress degradation conditions. Specifically, in the base and oxidative stress degradation conditions, the degradation peak was primarily attributed to significant degradation. Consequently, LC-MS was utilized for basic and oxidative degradation solely for the proposed molecular structure and fragmentation pathways. For conducting LC-MS studies of IMEG under diverse stress conditions were performed utilizing a mobile phase made up of 10 mM ammonium format in methanol (75:25, v/v) at pH 3.0, adjusted with formic acid. Degradation patterns were assessed by analyzing peak areas and comparing them with those of the standard. In photolytic, acidic, and thermal stress degradation conditions, the additional significant peak of degradants was not observed. However, there was a reduction in the intensity of the chromatogram of IMEG was observed by 1.63%, 1.25%, and 7.7%, in acidic, thermal, and photolytic stress conditions, respectively. Significant degradation was observed after applying basic and oxidative stress conditions, indicated by distinct degradation peaks as shown in Fig.-3 to 4.

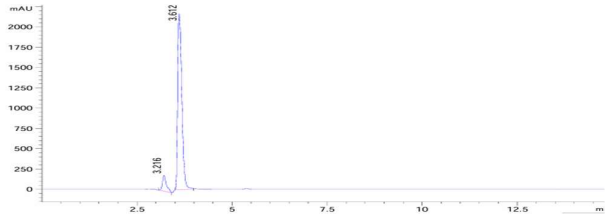

Fig.-3: Chromatogram of Imeglimin Hydrochloride after Applying Basic Stress Conditions

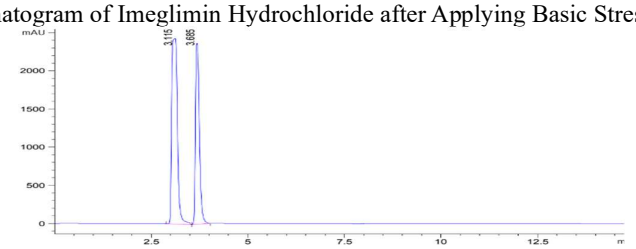

Fig.-4: Chromatogram of Imeglimin Hydrochloride after Applying Oxidative Stress Conditions

Analytical Method Validation

The developed method for stability studies of IMEG underwent rigorous validation across various analytical parameters. The detector exhibited excellent peak purity of IMEG, indicating the specificity of the developed method. Accuracy studies were conducted by calculating the % recovery of standard IMEG added to pre-analyzed commercial formulations containing IMEG. The accuracy was determined to be 99.103 ± 0.51% for the standard drug. Additionally, the validation parameters included a drug linearity of 0.9988 within the specific range of 50-250 µg/ml. The limits of detection (LOD) and

quantification (LOQ) were found to be 10.9 µg/ml and 33.31 µg/ml, respectively. Intraday and interday precision values were calculated as 0.76 and 0.95, respectively. The theoretical plates and tailing factor were measured at 6238 ± 1.769 and 1.324 ± 0.971, respectively.

MS Study of IMEG **Along with Proposed Degradation Pathway of** IMEG

The optimized chromatographic conditions were fine-tuned to ensure the precise separation of IMEG and its degradation products, providing perfect specificity with LC-MS. Additionally, the source conditions were optimized to improve signal strength and sensitivity as per the specific conditions mentioned for MS. Figure-5 presents the high-resolution mass spectra of the standard IMEG.

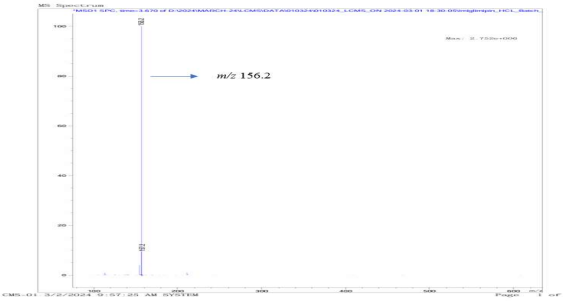

Fig.-5: Mass Spectra of Standard Imeglimin Hydrochloride

As mentioned, the highest degradation along with the clear additional degradants peak was observed in basic and oxidative stress conditions for which the characterization of degradation peak was done using high-resolution mass spectrometry. The typical mass spectra of the degradation sample of IMEG after basic degradation are represented in Fig.-6 below.

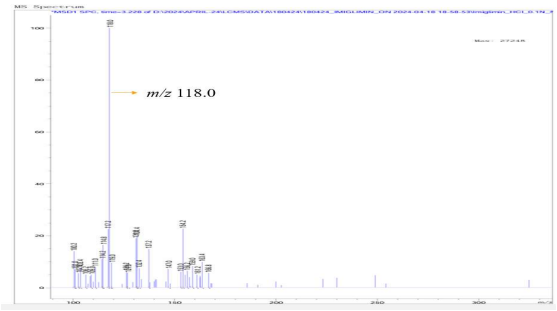

Fig.-6: Mass Spectra of Imeglimin Hydrochloride Along with its Degradation Product After Applying Basic Stress Conditions

The observed m/z values for the molecular ion peak and major fragments (base peak) of the base degradation products were m/z 156 and m/z 118, respectively. These values were matched with the molecular weight and fragmentation scheme of the degradation product of the drug. It was known that IMEG underwent base hydrolysis to give a base degradant product in the presence of NaOH. The degradation route was explained based on the fact that the standard IMEG mass spectra showed m/z 156 and the degrading mass spectra showed m/z 118. Therefore, these ratios could have been found if ethanamine had been separated as a fragment. Thus, the proposed fragmentation pathway for IMEG degradation product (DP-1) is illustrated in Fig.-7 below:

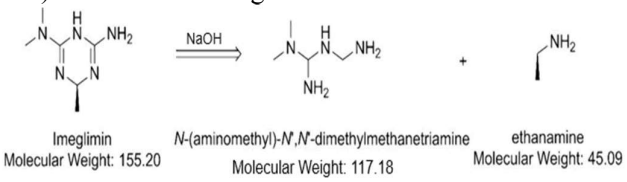

Fig.-7: Proposed Fragmentation Pathway of Imeglimin Hydrochloride Degradation Product (DP-1) after Applying Basic Stress Conditions

Similarly, significant degradation was observed in the form of an additional degradation peak after applying oxidative stress conditions. The mass spectra of IMEG following oxidative stress conditions are shown in Fig.-8 below.

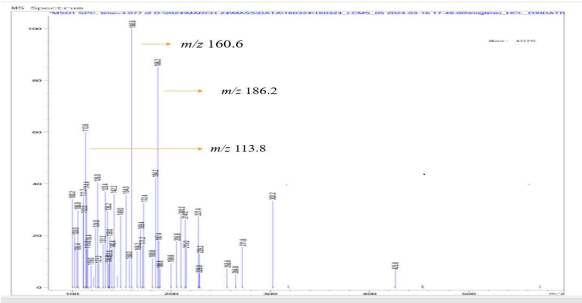

Fig.-8: The Mass Spectra of Imeglimin Hydrochloride Following Oxidative Stress Conditions

As observed in Fig.-8, multiple peaks were detected, suggesting the production of a variety of fragments following the application of oxidative stress conditions. However, the most likely fragmentation pathway of IMEG was determined by correlating with the most prominent and closely matching m/z values in the mass spectrum of IMEG after oxidative stress conditions were applied. The oxidation of IMEG resulted in the formation of this oxidative degradant product. The oxidation product had a molecular ion peak at m/z 160.6, with major fragment ions observed at m/z 188, 186, 172, 160, 158, 119, and 116 Da. Saturation of the triazine moiety in IMEG led to the formation of (6R)-N2, N2,6-trimethyl-1,3,5-triazinane-2,4-diamine, which exhibited a molecular ion base peak at m/z 160. This molecule could further fragment to produce a fragment at m/z 119. Additionally, oxidation of IMEG resulted in N-hydroxide and hydroxylamine derivatives at the 1- and 2-positions of the triazine moiety, with an m/z of 188. The formation of the hydroxylamine derivative was confirmed by the removal of water, yielding a fragment with m/z 172. Oxidation of IMEG could also lead to a nitro derivative with m/z 186. Furthermore, IMEG could fragment into m/z 158 and m/z 116. Thus, the proposed fragmentation pathway for IMEG degradation products after applying oxidative stress conditions is illustrated in Fig.-9 below.

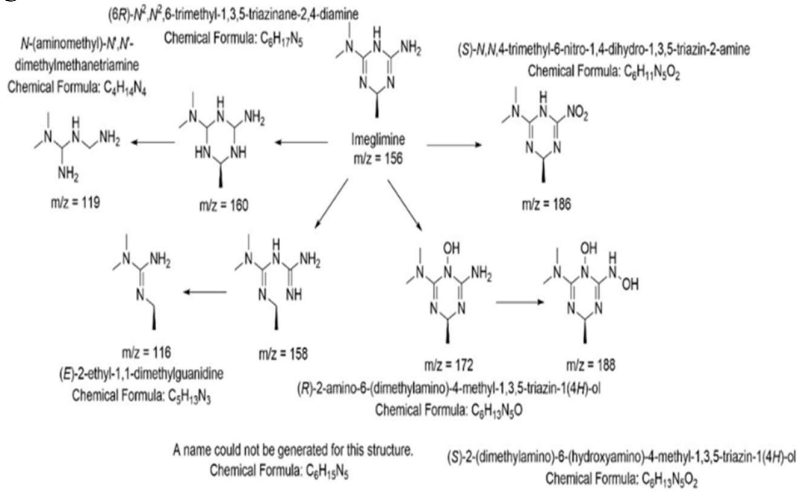

Fig.-9: Proposed Fragmentation Pathway of Imeglimin Hydrochloride Degradation Product after Applying Oxidative Stress Conditions

The peak purity and retention time of the degradation products of IMEG following exposure to basic and oxidative conditions are detailed in Table-2 below.

Table-2: Peak Purity and Retention Time of Degradation Products of Hydrochloride Following Exposure to Basic (B1) and Oxidative (O1) Conditions

Degradation product	*R_t (min)	Resolution factor	Tailing factor
B_1	3.216	1.23	1.56
O_1	3.115	1.35	1.17

* Mean value of three determinations

The summarized data of degradation products of IMEG, including the respective m/z values of degradants and fragment ions, were presented in Table-3 as given below.

Table-3: Summary of High-Resolution Mass Spectrometric Data of Imeglimin Hydrochloride Along with the Degradation Products (DP) after Applying Basic (B1) and Oxidative (O1) Stress Conditions

Description	Observed mass $[M+H]^+$				Molecular formula $[M+H]^+$			
IMEG	156.2				$C_6H_{14}ClN_5$			
DP-1 (B1)	117.0				$C_4H_{14}N_4^+$			
DP-2 (O1)	186	188	172	160	$C_6H_{11}N_5O_2$	$C_6H_{13}N_5O_2$	$C_6H_{13}N_5O$	$C_6H_{17}N_5$
	158	119	116	-	$C_6H_{15}N_5$	$C_4H_{14}N_4$	$C_5H_{13}N_3$	-

CONCLUSION

The degradation profile of Imeglimin hydrochloride was investigated under various extreme conditions, including acidic, oxidative, basic, photolytic, and thermal environments. The study utilized an LC-MS technique to effectively differentiate all degradation products of Imeglimin hydrochloride under these conditions. Significant degradation was seen during base and oxidative stress conditions in the form of additional peaks of degradants, while the compound remained stable with minimal degradation under thermal, acidic, and photolytic stress conditions. The developed LC-MS method was validated, and a detailed degradation pathway for Imeglimin hydrochloride was presented under specific stress conditions. Overall, this research provides valuable insights into the forced degradation studies and the assessment of degradation products of Imeglimin hydrochloride using an advanced and highly sensitive chromatography-mass spectrometry tool.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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